Monitoring Progress Report for Deepwater Horizon NRDA Phase 1 Early Restoration Project

MISSISSIPPI ARTIFICIAL REEF HABITAT PROJECT

Prepared by Mississippi Department of Environmental Quality

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INTRODUCTION

The Mississippi Trustee, Mississippi Department of Environmental Quality, and the other *Deepwater Horizon* Trustees selected the Mississippi Artificial Reef Habitat Project as a Phase 1 *Deepwater Horizon* early restoration project to partially compensate the public for injury to secondary productivity in the Mississippi Sound. This document includes a summary of the sampling methods to monitor the artificial reef sites and project-specific monitoring data results of biomass and secondary production on reef sites. This report is a summary of the first year of sampling.

PROJECT OVERVIEW

The Mississippi Artificial Reef Habitat project deployed nearshore artificial reefs in the Mississippi Sound. Nearshore artificial reefs provide valuable hard bottom habitat with foraging and shelter sites for various species of larvae and sessile epifauna and infauna. There are 67 existing nearshore artificial reef areas that are each approximately 3 acres in size. Approximately half of these existing reef areas have a low profile and consist of crushed concrete or limestone. With the Mississippi Artificial Reef Habitat project, approximately 100 acres of crushed limestone was added to 47 existing reef areas or hard substrate habitats indicated in Figure 1. The resulting artificial reefs consist of low profile reefs 4 to 6 inches above the seafloor.

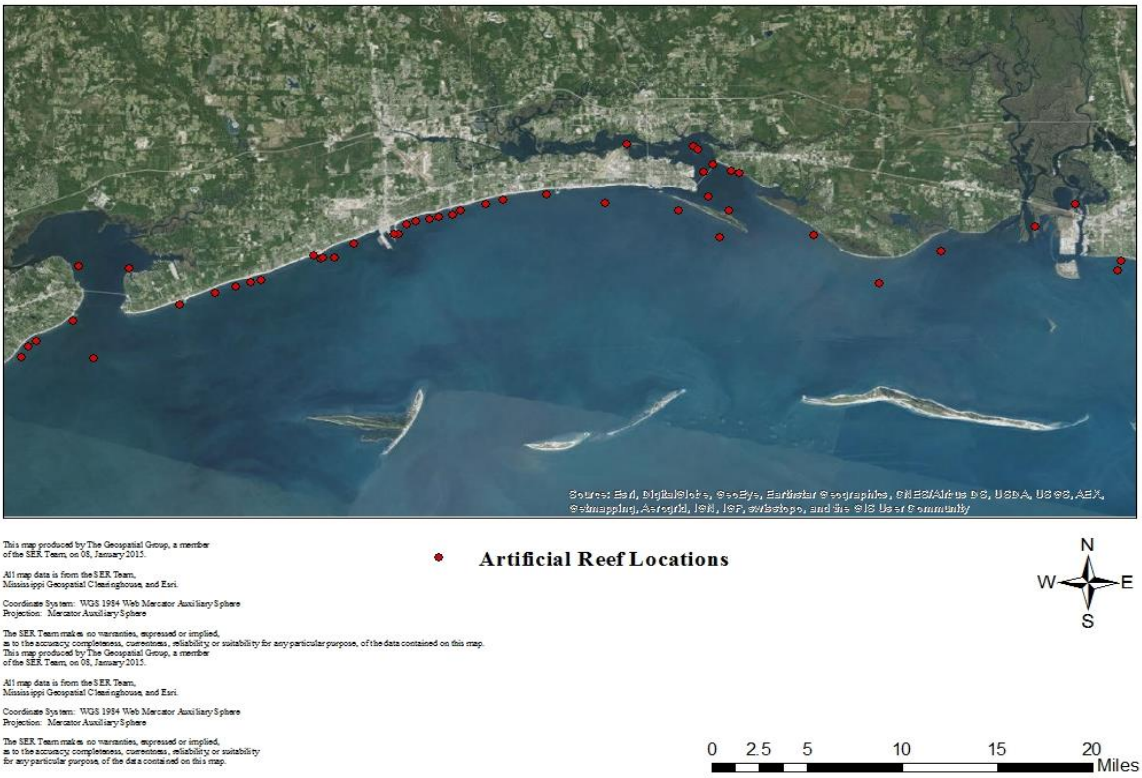

Figure 1. The 47 artificial reef habitat locations that were enhanced by this project.

RESTORATION OBJECTIVES AND PERFORMANCE CRITERIA

The goal of the project was to create artificial reefs to support secondary production. The specific objectives were to 1) Create or enhance existing nearshore artificial reefs (Figure 1) that are sustained for the expected lifespan of the project; and 2) Promote habitat utilization of reefs by mobile and sessile invertebrate infauna and epifauna.

Performance criteria are being used to determine restoration success.

- Reefs are deployed as designed in designated areas (as-built)
- At year 1 and 2, the [average] biomass of the non-bivalve epifauna and infauna on the reef should be at least 84g ww/m^2.

METHODS

To address the first performance criteria, as-built surveys were conducted. In the fall of 2012, approximately 4,000 cubic yards of cultch material (#56 and #57 domestic limestone) were deployed to enhance five artificial reefs. In spring of 2013, 25,000 cubic yards of cultch material (#56 and #57 domestic limestone) were deployed to and enhance 42 artificial reefs. The GPS locations of these reefs were confirmed post-deployment in contractor reports (CCE 2012; CCE 2013).

To address the second performance criteria, the contractor followed the standard operating procedures (SOP) provided in Appendix 1. In summary, secondary production (specifically, epifauna) was monitored using settlement substrate baskets and settlement trays. Analysis of substrate baskets included density (#/m^2) and diversity measures (# of species / m^2) of various organisms. In addition, biomass measures were performed to provide estimates of secondary productivity at each of the monitoring sites. Settlement trays served as supplementary gear to assess colonization and utilization by reef-associated mobile macrofauna and were analyzed for abundance, diversity and associated size metrics of macrofaunal species.

Four sites including one control site (Oak Street Reef) were monitored (Figure 2). In May 2014, baskets and trays were deployed and then retrieved approximately 6-weeks later. A total of twenty-four baskets and sixteen trays were deployed across the four sites.

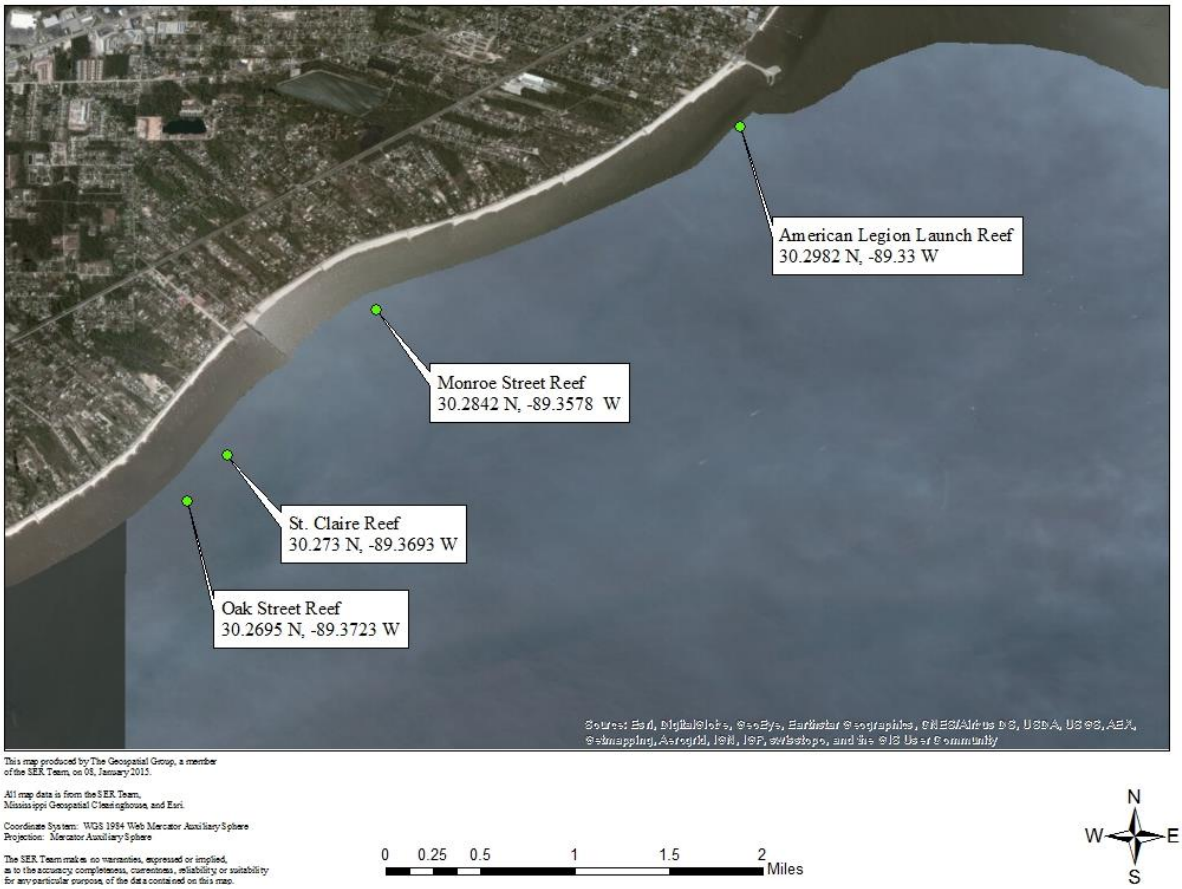

Figure 2. Monitoring sites for Artificial Reef Habitat Project. Oak Street Reef is a control site.

RESULTS

Results towards the performance criteria are reported in Table 1.

Table 1: Performance target and actual measurements for each performance criteria

Performance criteria	Pre-construction	Post-construction target	Post-construction actual	Year 1 target	Year 1 actual
As-built survey	Reefs	47 reefs enhanced	47 reefs enhanced	n/a	n/a
Epi-faunal and infaunal species comp, density and biomass (average across treatment reefs)	n/a	n/a	n/a	At least 84 g ww/m^2	TRAYS =250 g ww/m^2 BASKETS = 197 g ww/m^2 -average across treatment sites

Table 2 represents all invertebrate taxa identified from baskets at all of the artificial reef sampling locations. A total of 39 taxonomic groups were identified. Five taxonomic groups were recorded as presence/absence and were not quantified. Data from individual gear were compiled by reef for analysis. Organisms that far exceeded the mesh size of the gear were excluded from analysis (NA). Reef abbreviations are as follows: AL = American Legion; MS = Market Street; SC = St. Claire; OS = Oak Street.

Table 2: Basket biomass summary

ALL Reef Taxa in Baskets	AL Reef Total # Orgs	AL Reef Total Wet Wt.(g)	AL Total Production dry wt ug/day	MS Reef Total # Orgs	MS Reef Total Wet Wt.(g)	MS Total Production dry wt ug/day
Bryozoa	Present			Present		
***Balanus* sp.**	Present			Present		
Hydrozoa	Present			Present		
***Argulus* sp.**	Present			Present		
Ascidean	Present					
Nereidae	1659	9.16	64319.74	2091	11.84	46198.94
***Polydora* spp**	54	0.010	84.36	92	0.02	149.92
Streblospio gynobranchiata	1	0.0003	2.00	1	0.0005	3.39
Eteone Heteropoda						
Oligochaeta						
Mytilidae mussel	106	0.64	2157.62	138	0.34	1344.51
Mytilopsis leucophaeata	1	0.002	12.03	4	0.02	60.73
Crassostrea virginica	1	0.0002	1.827	1	0.0001	1.03
Opisthobranchia	19	0.018	99.93	12	0.02	114.59
Nudibranch sp.B	9	0.002	14.26	45	0.009	76.38
Bivlave	1	0.0003	2.32			
Hydrobiidae	1	0.0003	2.16			
Macoma mitchelli	2	0.0006	4.14			
Mulinea lateralis						
Turbellaria	171	0.09	563.03	216	0.13	740.2
Sipunculida				1	0.005	22.02
Eurypanopeus depressus	846	89.47	199159.39	697	81.28	178336.89
Rhithropanopeus harrisii	35	0.60	1940.91	215	4.094	13000.13
Panopeidae	105	0.15	730.54	491	0.54	2767.48
Panopeus simpsoni	2	1.45	2242.75	5	7.34	9745.75
Menippe adina						
Brachyuran megalopae	45	0.02	113.09	83	0.02	182.31
***Palaemonetes* sp.**	377	37.69	87154.11	276	26.858	62486.06
Macrobranchium ohioae	32	0.21	810.68	98	1.01	3556.22
Caridean						
***Apocorophium* spp**	44	0.02	131.57	23	0.01	74.98
***Hourstonius* sp.**				2	0.0002	1.89
Decapoda				3	0.004	20.78
Clibanarius vittatus	1	2.78	3322.03			
Callinectes sapidus				NA	NA	NA
Insecta	1	0.0002	1.67			
Colembolla						
Brachyuran zoea						
Melitidae amphipods	11370	15.92	87088.35	11583	17.37	93861.26

Table 2: Basket biomass summary (Con't)

ALL Reef Taxa in Baskets	SC Reef Total # Orgs	SC Reef Total Wet Wt.(g)	SC Total Production dry wt ug/day	OS Reef Total #Orgs	OS Reef Total Wet Wt.(g)	OS Total Production dry wt ug/day
Bryozoa	Present			Present		
***Balanus* sp.**	Present			Present		
Hydrozoa				Present		
***Argulus* sp.**	Present			Present		
Ascidean						
Nereidae	2667	14.13	89978.75	4155	22.91	89807.79
***Polydora* spp**	165	0.03	212.83	754	0.12	1022.14
Streblospio gynobranchiata	3	0.001	10.19	19	0.004	36.17
Eteone Heteropoda				1	0.0001	1.39
Oligochaeta				3	0.0004	4.16
Mytilidae mussel	224	1.28	4482.69	812	2.32	8981.62
Mytilopsis leucophaeata	1	0.002	12.03	4	0.009	44.43
Crassostrea virginica	1	0.0002	1.82	1	0.0002	1.82
Opisthobranchia	46	0.07	372.60	168	0.35	1763.70
Nudibranch sp.B	94	0.02	136.89	222	0.039	320.73
Bivlave				1	0.00006	0.67
Hydrobiidae				26	0.007	54.80
Macoma mitchelli						
Mulinea lateralis				1	0.003	14.22
Turbellaria	250	0.09	590.81	370	0.27	1523.42
Sipunculida						
Eurypanopeus depressus	280	55.88	110889.66	226	37.41	76641.54
Rhithropanopeus harrisii	781	18.62	56297.92	747	22.11	63301.36
Panopeidae	708	0.60	3373.71	913	0.86	4675.69
Panopeus simpsoni	29	20.42	29652.31	1	0.31	578.25
Menippe adina	1	0.66	1052.08			
Brachyuran megalopae	282	0.09	640.07	252	0.07	564.097
***Palaemonetes* sp.**	320	27.18	64525.76	414	40.50	93832.76
Macrobranchium ohioae	9	0.07	260.71	30	0.31	1089.032
Caridean				2	0.02	83.64
***Apocorophium* spp**	192	0.08	541.44	161	0.07	515.23
***Hourstonius* sp.**						
Decapoda	1	0.0009	5.74	7	0.001	14.51
Clibanarius vittatus						
Callinectes sapidus	NA	NA	NA	NA	NA	NA
Insecta	1	0.0002	1.70			
Colembolla				1	0.0001	1.02
Brachyuran zoea				6	0.003	21.19
Melitidae amphipods	15037	20.01	110190.39	5296	6.835	37917.01

Table 3 represents invertebrate and vertebrate taxa identified from trays at all artificial reef sampling locations. A total of 15 taxonomic groups were identified. Data analyses were conducted on a per reef basis. Reef abbreviations are as follows: AL = American Legion; MS = Market Street; SC = St. Claire; OS = Oak Street.

Table 3: Tray biomass and productivity summary

All reef taxa in trays	**AL Reef # Orgs**	**AL Reef Wet Wt. (g)**	**AL Reef Production (g) AFDW / day**	**MS Reef # Orgs**	**MS Reef Wet Wt. (g)**	**MS Reef Production (g) AFDW / day**
Archosargus probatocephalus	-	-	-	-	-	-
Bairdiella chrysoura	-	-	-	-	-	-
Callinectes sapidus	-	-	-	-	-	-
Chaetodipterus faber	-	-	-	1	2.9	0.014
Chasmodes bosquianus	1	0.3	0.003	1	0.4	0.003
Chloroscombrus chrysurus	-	-	-	-	-	-
Gobiesox strumosus	13	4.8	0.043	8	3.8	0.028
Gobiosoma bosc	7	1.5	0.0153	3	1.3	0.010
Hypsoblennius hentz	1	1.6	0.0093	-	-	-
Hypsoblennius ionthas	4	2	0.016	13	11.1	0.072
Lagodon rhomboides	2	20.1	0.063	-	-	-
Menippe adina	-	-	-	-	-	-
Opsanus beta	8	320.4	0.654	2	74.2	0.158
Palaemonetes vulgaris	4	1	0.031	2	0.5	0.015
Panopeidae	53	27.6	0.723	75	50.3	1.23
Total	**93**	**379.3**	**1.55**	**105**	**144.5**	**1.53**

All reef taxa in trays	**SC Reef # Orgs**	**SC Reef Wet Wt. (g)**	**SC Reef Production (g) AFDW / day**	**OS Reef # Orgs**	**OS Reef Wet Wt. (g)**	**OS Reef Production (g) AFDW / day**
Archosargus probatocephalus	-	-	-	1	2.2	0.011
Bairdiella chrysoura	10	20.6	0.103	-	-	-
Callinectes sapidus	2	456.2	3.54	2	32.4	0.433
Chaetodipterus faber	3	7.7	0.038	2	6.4	0.029
Chasmodes bosquianus	1	0.5	0.004	1	2.8	0.013
Chloroscombrus chrysurus	-	-	-	1	0.7	0.005
Gobiesox strumosus	6	11.7	0.055	7	16.9	0.081
Gobiosoma bosc	3	2	0.014	5	5	0.031
Hypsoblennius hentz	1	0.7	0.005	-	-	-
Hypsoblennius ionthas	9	7.9	0.053	7	10.1	0.059
Lagodon rhomboides	-	-	-	-	-	-
Menippe adina	-	-	-	1	1.8	0.037
Opsanus beta	1	7	0.025	5	100.8	0.249
Palaemonetes vulgaris	-	-	-	10	2.2	0.069
Panopeidae	65	45.4	1.12	62	50.8	1.179
Total	**91**	**539.1**	**4.96**	**104**	**232.1**	**2.20**

Table 4 represents invertebrate and vertebrate taxa identified from trays at all artificial reef sampling locations. Density and diversity of identified taxa were calculated per sampling site. Reef abbreviations are as follows: AL = American Legion; MS = Market Street; SC = St. Claire; OS = Oak Street.

Table 4: Tray taxa density and diversity

AL Identified Taxa List	AL Taxa Count	MS Identified Taxa List	MS Taxa Count
Archosargus probatocephalus	-	*Archosargus probatocephalus*	-
Bairdiella chrysoura	-	*Bairdiella chrysoura*	-
Callinectes sapidus	-	*Callinectes sapidus*	-
Chaetodipterus faber	-	*Chaetodipterus faber*	1
Chasmodes bosquianus	1	*Chasmodes bosquianus*	1
Chloroscombrus chrysurus	-	*Chloroscombrus chrysurus*	-
Gobiesox strumosus	13	*Gobiesox strumosus*	8
Gobiosoma bosc	7	*Gobiosoma bosc*	3
Hypsoblennius hentz	1	*Hypsoblennius hentz*	-
Hypsoblennius ionthas	4	*Hypsoblennius ionthas*	13
Lagodon rhomboides	2	*Lagodon rhomboides*	-
Menippe adina	-	*Menippe adina*	-
Opsanus beta	8	*Opsanus beta*	2
Palaemonetes vulgaris	4	*Palaemonetes vulgaris*	2
Panopeidae	53	*Panopeidae*	75
Total	**93**	**Total**	**105**
# of Species	**9**	**# of Species**	**8**
# of Trays	**4**	**# of Trays**	**4**
Taxa Density (#/m2)	**93 per m2**	**Taxa Density (#/m2)**	**105 per m2**
Taxa Diversity (#taxa/m2)	**9 per m2**	**Taxa Diversity (#taxa/m2)**	**8 per m2**
Tray = 0.25m2		Tray = 0.25m2	

OS Identified Taxa List	OS Taxa Count	SC Identified Taxa List	SC Taxa Count
Archosargus probatocephalus	1	*Archosargus probatocephalus*	-
Bairdiella chrysoura	-	*Bairdiella chrysoura*	10
Callinectes sapidus	2	*Callinectes sapidus*	2
Chaetodipterus faber	2	*Chaetodipterus faber*	3
Chasmodes bosquianus	1	*Chasmodes bosquianus*	1
Chloroscombrus chrysurus	1	*Chloroscombrus chrysurus*	-
Gobiesox strumosus	7	*Gobiesox strumosus*	6
Gobiosoma bosc	5	*Gobiosoma bosc*	3
Hypsoblennius hentz	-	*Hypsoblennius hentz*	1
Hypsoblennius ionthas	7	*Hypsoblennius ionthas*	9
Lagodon rhomboides	-	*Lagodon rhomboides*	-
Menippe adina	1	*Menippe adina*	-
Opsanus beta	5	*Opsanus beta*	1
Palaemonetes vulgaris	10	*Palaemonetes vulgaris*	-
Panopeidae	62	*Panopeidae*	65
Total	**104**	**Total**	**91**
# of Species	**12**	**# of Species**	**10**
# of Trays	**4**	**# of Trays**	**3**
Taxa Density (#/m2)	**104 per m2**	**Taxa Density (#/m2)**	**91 per 0.75m2**
Taxa Diversity (#taxa/m2)	**12 per m2**	**Taxa Diversity (#taxa/m2)**	**10 per 0.75m2**
Tray = 0.25m2		Tray = 0.25m2	

DATA

All data from basket and tray samples are being stored in the Center for Fisheries Research and Development, Oceanography and Caylor buildings at the Gulf Coast Research Lab.

Baskets

Sampling in 2014 yielded 23 of 24 baskets recovered and processed. Field gear was lost either to weather or tampering/removal by boat activity. Processing of basket samples is complete, and sample sorting and taxonomic identification have all passed QA/QC requirements. Identified organisms have been measured and weighed. Wet weight is determined from actual organism wet weight, volumetric conversion to wet weight, dry weight conversion to wet weight, and regression derivation of wet weight depending on size and taxon of organisms. The numbers of organisms for each taxon were summed for all gear at an individual reef. Total wet weight was converted to total ash free dry weight and then to secondary production rate using standard conversion factors. Data File "Artificial Reef Data Basket Summary" provides information (list of species, # of each species, the weight of each species and secondary production rate of each species) for each individual basket collected as well as summed data for each artificial reef sampling location. The data file "Artificial Reef Spring 2014 - Baskets" summarizes data at each reef and provides average secondary production rates for each artificial reef sampling location.

Trays

Sampling in 2014 resulted in 15 of 16 trays being recovered and processed. Field gear was lost either to weather or tampering/removal by boat activity. Data from all trays have been compiled, fully processed and have passed QA/QC requirements. Biomass of identified organisms was recoded as wet weight and then converted to ash free dry weight and then secondary production rate through standard conversion factors and equations (Edgar, 1990; Edgar and Shaw, 1995; Brey, 2001; Brey et al., 2010). Data File “Artificial Reef Data Spring 2014 - Tray” provides a summary of the data (list of species, # of each species, weight of each species and secondary production rate of each species) for individual trays and for each artificial reef location; as well as a summary of average secondary production rates for each artificial reef location.

SUMMARY

Table 5 shows summed values for number of organisms, wet weight(g), and secondary production [dry wt(ug)/day] and are presented for each of the four artificial reef sites. Also presented by reef are the per basket averages for number of organisms, wet weight(g), secondary production[dry wt(ug)/day] and secondary production per square meter per day.

Table 5: Summary of secondary production rates using basket data

	Oak St. Reef TOTAL (n=6 baskets)	**St. Claire Reef** TOTAL (n=6 baskets)	**Monroe St. Reef** TOTAL (n=6 baskets)	**American Legion Reef** TOTAL (n=5 baskets)
Total # orgs	14593	21092	16077	14883
Total wt.(g)	134.60	159.21	150.91	158.23
Total production dry wt ug/day	382812.51	473230.15	412745.47	449958.53
Avg#orgs/basket	2432.17	3515.33	2679.5	2976.67
Avg Wt(g)/basket	22.43	26.53	25.152	31.65
Avg Prod (ug dry wt/day)/basket	63802.08	78871.69	68790.91	89991.71
Avg Prod (g dry wt/day)/m2	**0.46**	**0.57**	**0.50**	**0.65**

Table 6 shows the summed values for the number of organisms, wet weight(g), and secondary production [g AFDQ/day] for each artificial reef site. Also presented by reef are the per tray average for number of organisms, wet weight (g), and secondary production per square meter per day.

Table 6: Summary of secondary production rates using tray data

	Oak St. Reef TOTAL (n=4)	**St. Claire Reef** TOTAL (n=3)	**Monroe St. Reef** TOTAL (n=4)	**American Legion Reef** TOTAL (n=4)
Total # orgs	104	91	105	93
Total wt.(g)	232.1	539.1	144.5	379.3
Total production g AFDW/day	2.197	4.96	1.53	1.55
Avg#orgs/tray	26	1	26.25	23.25
Avg Wt(g)/tray	58.03	179.7	36.12	94.83
Avg Prod (g AFDW/day)/tray	0.55	1.65	0.38	0.39
Avg Prod(g AFDW/day)/m2	**2.20**	**6.61**	**1.53**	**1.55**

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APPENDIX 1

SOP -Field Gear Handling / Sample Collection

Basket Deployment

At each artificial reef study site, six baskets of reef substrate are placed at predetermined GPS starting positions throughout the study area within existing arrays of restored reef substrate radiating from the central site location, with exact locations determined by the presence of acceptable bottom substrate/confirmed reef material. Using a pole to probe the bottom, placement of baskets into soft mud is avoided. Before placing a basket, the settlement plate, buoy line and brick weight are attached. Each basket is slowly lowered in a horizontal orientation to the bottom from the side of a small vessel.

Tray Deployment

At each study site, four trays of reef substrate are placed along each of four radiating 36 m transects as described above for baskets, and as determined by the presence of acceptable bottom substrate (i.e., confirmed reef material). Using a pole to probe the bottom, placement of baskets into soft mud is avoided. Each tray is slowly lowered in a horizontal orientation to the bottom from the side of a small vessel.

Basket Retrieval

Baskets are retrieved approximately six weeks after deployment. Each basket is retrieved via its float line, and before the basket breaks the water surface, a large dip net (0.333mm mesh) is positioned under it to retain organisms which might be lost when retrieved. The basket is placed directly into a plastic tub to prevent sample loss as water drains from the substrate. Substrate rocks and associated biota are transferred from the basket to a labeled 5 gallon bucket where they remain submerged in seawater during transport for land-based processing. Live samples are kept as cool as possible to reduce organism mortality while transport for land-based processing.

Tray Retrieval

Trays are retrieved approximately six weeks after deployment. Each tray is retrieved via its float line. The rocks/organisms in the tray are transferred to a labeled 5 gallon bucket using a funnel shaped mat. Tray sample substrate is submerged in seawater during transport for land-based processing.

SOP – Tray Processing

Sample Handling

Lab processing of tray samples begins by using a 1/2 inch sieve, constructed of the same mesh screening used to line the settlement trays, to sieve and rinse the contents (substrate and biota) from each bucket. All organisms retained by the sieve are collected for laboratory analysis. Samples are stored in re-sealable bags and labeled

internally and externally with: collection date, site location, and tray identification number. All tray samples are frozen for storage and later work up.

Sample Identification

Tray samples are removed from the freezer and allowed to thaw completely prior to being examined. Each sample is then sorted by a technician in preparation for identification. Contents (finfish and macro -invertebrates) from each sample are identified to the lowest practical taxonomic level through the use of taxonomic keys and available literature. The mass of each organism (blotted wet weight) is taken using a digital bench scale. Samples are put back in the freezer to await QA/QC procedures.

SOP - Basket Processing

Sample Handling / Preservation

As soon as possible after retrieval (2-4hr), basket samples in 5 gallon buckets are brought to GCRL for extraction of organisms before preservation. A sample is processed by placing approximately 1/2 the contents of substrate/biota from the bucket onto mesh sieves (13mm) suspended above a wooden sorting board (repeated for remaining 1/2 of sample). A large plastic tub is placed under the open funnel end of the board to receive all organisms. Substrate rocks are spread over the suspended sieves for inspection and then washed and visually inspected to assure organisms are being collected into the wash tubs. Any live fish or large invertebrates are put into a labeled jar with seawater and placed on ice to anesthetize them before preservation.

Sorting

Samples must be sorted to remove and categorize organisms. A small spoonful of sample is examined in a gridded Petri-dish. Each dish is examined using a dissection microscope and all organisms are removed from the dish, by searching square by square on the dish grid. Organisms are placed into designated vials corresponding to major Phyla (Annelida, Arthropoda, Mollusca, and miscellaneous). A dish is examined twice without finding any organisms before it is considered sorted. Vials of sorted organisms are retained to await taxonomic identification.

Taxonomic Identification / Measuring

All organisms are identified to the lowest practical taxonomic level using taxonomic keys and available literature. Organisms within each taxon are divided up for estimating their biomass using a series of size-fractioning grids: 8.0mm, 6.0mm, 4.0mm, 3.0mm, 2.0mm, 1.5mm, 0.75mm and 0.5mm under a dissection microscope. Larger designated crustaceans are measured consistently (e.g., carapace width/length) using a ruler or calipers to the nearest mm. Organisms too large to be measured with grids are directly weighed. Size fractioned and enumerated data are recorded. Identified samples are returned to await determination of biomass estimates.

Biomass Estimates

Estimating biomass entails either directly taking wet weights or taking linear measurements of larger organisms to estimate wet weights via regression. However, biomass estimates for most organisms are obtained using image

analysis (MetaVue software) to convert compressed areas of known numbers of size-sorted organisms to volumes, and then to wet weights. Taxa with excessive organisms (>> 100) are subsampled for identifying and estimating biomass.

Image Analysis

Slide Mounting - There are two calibrated sets of slides representing different volume capacities: one for organisms in smaller size fractions, and the other set for larger size fractions. The contents of an individual taxon-size fraction (taxon specific and sized) are compressed ('squashed') by placing them between the top and bottom slides. See-saw like play in the top plate indicates that either the larger volume set should be used, or a wet weight should be directly taken. The somatic portion of hard-shelled organisms such as bivalves and gastropods is removed from the shell before being weighed.

Area Determination - For each slide squash, an image is acquired within MetaVue 7.1 software to determine the area of the compressed organisms. The area is determined by tracing the perimeter of each squashed image using the regions measurement tool. Using known magnification keys, Metavue calculates the area of the image. Volume estimates are then obtained from the area measurements using the appropriate conversion factor for the calibrated set of squash plates.

Wet Weight

Larger organisms are weighed using an electronic balance. Organisms are gently blotted dry and weighed (nearest 0.00001 g). Abundant large crustaceans (i.e. crabs, grass shrimp) are directly measured using dial calipers, or a millimeter ruler.

Regressions

For predominant taxa (i.e., taxa represented by multiple size fractions that are common within a large percentage of the samples), linear regressions of per individual biomass versus size fraction on a log-log scale enable volume conversions based on counts within any respective size fraction for that taxon. Wet weights of individual large crustaceans which have been measured are also typically obtained though established length-weight regressions.

SOP -Quality Assurance / Quality Control

Sorting

All samples are tracked for each sorter, and one sample is selected from a set of 10 using a random number generator. The selected QC sample is re-sorted by an experienced sorter and any whole organisms are removed and saved. For each QC sample, sorting efficiency is calculated using the following formula:

$$\frac{\text{\# of organisms originally sorted}}{\text{\# of organisms originally sorted}} \times 100$$

# of organisms originally sorted + additional # found in resort

Samples require a 90% numeric efficiency to pass QC. If the QC does not pass, problem areas (i.e. failure to recognize/remove specific taxon) are reviewed with the sorter and all 10 samples in the set are resorted.

Taxonomic Identification (Baskets and Trays)

All samples are tracked for each sorter, and one sample is selected from a set of 10 using a random number generator. Organisms from the selected QC sample are re-identified and re-counted by a senior taxonomist. The QC should be done in a timely manner so that subsequent processing (i.e. biomass) may proceed. As each taxon is identified and counted during the re-check, results should be compared to the original identification data sheet. After QC, any changes in number and/or species identification should be noted and changed in the database. Accuracy for taxonomic QC is determined using the following formula:

$$\frac{\text{Total \# organisms in original sample} - \text{Total \# errors}}{\text{Total \# organisms in QC recount}} \times 100$$

Errors include:

1) Counting Error (i.e. recording 11 individuals when only 10 are present)

2) Identification Error (i.e. ID species X as species Y, when both are present)

3) Unrecorded Taxa Error (i.e. do not identify species X when it is present)

Taxonomic samples require 90% accuracy to pass QC. If accuracy is less than 90%, the technician is advised of problem areas and the entire set of 10 samples is re-identified and re-counted.

Data Management

The goal of QC for data management is to correct/remove any erroneous individual data points as well as to correct/remove inconsistencies that jeopardize the integrity of the database. All data entry and transcription from field and laboratory bench sheets is verified. Data are verified by personnel not involved in the data entry, so that individuals are not checking the same data he/she entered. Initials of persons performing data entry and data verification are documented. Once a set of samples passes the QC criteria, a worker (other than the original person) performs a check of the raw data sheets to ensure that the taxonomic codes are correct. Any inconsistencies found between taxon codes and species identification are noted and discussed with the taxonomist to determine if correction is required for the code or for the species. Once the code check is complete, the taxonomic raw data sheet is initialed and dated. Data are entered into appropriate spreadsheets, which are dated and initialed by the person entering data. Following the initial entry of data into the computer database, a 100% manual recheck of data is performed (dated and initialed by person performing re-check). Any errors found in the

data during the re-check (i.e. numeric and/or typographic) are noted on the Data QC Sheet. Corrections are tracked on a Data Management log to show original entry, corrected entry and the initials and date of the person making database corrections. To reduce the threat of data loss, all data stored on laboratory computers are frequently backed up on a back-up computer and on a USB jump drive designated for the project.

* (Sorting, Biomass and Taxonomic Identification *QA/QC* procedures are adapted from guidelines of the EPAEMAP Estuaries 1992 Louisiana Province Quality Assurance Project Plan *EPA/600/x92/xxx* and from the EPA EMAP Estuaries 1995 West Indian Province Quality Assurance Project Plan) **